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Angiopoitin-2/Angiopoitin-1 Ratio in Acute Leukemia Patients with Febrile Neutropenia

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ABSTRACT

Background: Sepsis is a major cause of death in acute leukemia patients presented with febrile neutropenia (FN) despite the current use of modern antibiotics and resuscitation therapies. Septic shock is known to be one of the worst complications of sepsis. It frequently precedes multi-organ dysfunction syndrome and death. Septic shock Biomarkers can have an important place in predicting the severity of sepsis. Breakdown of the endothelial barrier is the hallmark of septic shock. Proteins that physiologically regulate endothelial barrier integrity as Angiopoitin-2 (Ang-2), Angiopoitin-1 (Ang-1) and vascular endothelial growth factor (VEGF) are thought to be candidate biomarkers of septic shock development.

Objectives: The aim of the present work was to evaluate the value of Ang-2/Ang-1 ratio as an early marker for prediction of occurrence of septic shock in acute leukemia patients with FN.

Patients and Methods: A prospective study was performed on inpatients with new onset FN from the Hematology Unit, Main University Hospital and Medical Research Institute. Levels of Ang-1 and Ang-2 were measured after the onset of neutropenic fever. Ang-2/Ang-1 ratio was calculated. Patients were categorized based on the occurrence of septic shock within 28 days as an outcome into two groups: Non-complicated FN patients (group A) and complicated FN patients (group B).

Results: High Ang-2/Ang-1 ratio was associated with septic shock development in acute leukemia patients (p= <0.001). Higher Ang-2/Ang-1 ratio was associated with lower Multinational Association for Supportive Care in Cancer (MASCC) score (p=<0.001) and higher CRP levels (p=0.011). Low Ang-1 and high Ang-2 levels were associated with higher mortality (p=0.035 & p=0.003).

Conclusions: High Ang-2 level and Ang-2/Ang-1 ratio are predictive of septic shock development in acute leukemia patients with febrile neutropenia. High Ang-2 and low Ang-1 levels in febrile neutropenic patients are markers of higher mortality.

Key Words: *Angiopoitin-1 – Angiopoitin-2 – Febrile neutropenia.*

INTRODUCTION

Febrile neutropenia (FN) frequently complicates chemotherapy in cancer patients, despite the intensive efforts in infection prevention. Myelosuppression, principally neutropenia, remains among the major toxicities of systemic cancer chemotherapy, particularly among patients with hematologic malignancies for which chemotherapeutic protocols are myelosuppressive by design. Septic shock can emerge as a fulminant complication, despite intensive treatment comprising broad-spectrum antibiotics and best supportive care **[1,2]**.

The septic response is an immensely complex cascade of events that involves inflammatory and anti-inflammatory processes, humoral and cellular reactions and circulatory abnormalities **[3]**. The establishment of sepsis diagnosis and evaluation of its severity is overwhelmed by the high variability and non-specificity of the signs and symptoms of sepsis **[4]**. Septic shock evolution is one of the worst complications of sepsis, frequently ending by multi-organ dysfunction syndrome and death. The Multinational Association for Supportive Care in Cancer (MASCC) prognostic model was introduced to help in predicting the clinical outcome of patients with FN **[5-7]**.

The fitness of inflammatory biomarkers in identifying patients with febrile neutropenia at high-risk of sepsis evolution continues to be explored; among them procalcitonin, C reactive protein, and others which are extensively studied **[8-10]**. The endothelial barrier integrity and the cellular and molecular mechanisms of its regu-

lation have been outlined where vascular endothelial growth factor-A (VEGF-A), Angiopoitin-1 (Ang-1), and Angiopoitin-2 (Ang-2) play pivotal roles in the physiological control of angiogenesis **[11]**.

Ang-1 has been shown to be a principal activator of the tyrosine kinase receptor Tie-2 (Tek) resulting in a downstream activation of the phosphatidylinositol 3'-kinase/Akt survival pathway, thereby promoting the survival of endothelial cells. Ang-2 is the typical antagonist of Ang-1 and hence, prevents Tie-2 activation. This action leads to vessel destabilization, an essential step in angiogenesis initiation by VEGF-A. Balanced and consecutive expression of angiopoietins and VEGF is needed for successful angiogenesis **[12,13]**.

Laboratory markers that can reliably early predict septic shock occurrence are deeply needed besides the clinical MASCC model. This need motorized many research efforts. In this regard, Ang-1 and Ang-2 were evaluated in the present work as possible early predictive markers for the occurrence of septic shock and mortality among acute leukemia patients with severe febrile neutropenia.

PATIENTS AND METHODS

Patients:

The present study was conducted on 36 patients diagnosed as acute leukemia with severe febrile neutropenia (FN) following induction or consolidation chemotherapy at the Hematology Unit, main University Hospital and Hematology department, Medical Research Institute, Alexandria University.

Inclusion criteria were: (1) Fever (T ≥38°C) for more than one-hour observation period or a single reading ≥38.3°C. (2) Chemotherapy-induced severe neutropenia (absolute neutrophilic count <0.5 x 10^9/L at the time of fever onset, and (3) Persistence of severe neutropenia until the time of blood sample collection. Exclusion criteria were: (1) Death during the study due to any condition other than septic shock, and (2) Chronic liver or kidney disease.

Patients were followed-up for a period of 28 days from the onset of FN. Sepsis diagnosis was established in the presence of two or more of the following: (1) Temperature ≥38°C for more than one hour. (2) Heart rate >90 beats/minute. (3) Respiratory rate >20 breaths/minute or PaCO2 <32mmHg. (4) A microbiologically proven or clinically evident source of infection. A diagnosis of septic shock was established when sepsis induced hypotension (systolic arterial pressure <90mmHg or dropping >40 mmHg from baseline) persisted despite adequate volume resuscitation. During the follow-up of patients, 10 patients died with a median survival time of 25.6 days (Fig. 1).

All subjects enrolled in this study signed a written informed consent before participation. Approval of the ethical committee of the faculty of medicine, Alexandria University was obtained. The procedures followed were according to the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Methods:

Acute leukemia patients with FN were stratified by calculation of the MASCC score at the onset of FN. Samples were withdrawn with the next routine sample collection requested for them. As per local protocol, the first routine sample collection requested for all admitted FN patients includes: Complete blood count (CBC), C reactive protein (CRP), liver function tests, kidney function tests and electrolytes. Blood and urine cultures were obtained and broad-spectrum antibiotics were initiated. All patients underwent chest radiography. Other imaging studies were performed when judged necessary by the attending physician.

Serum levels of Ang-1 and Ang-2 were measured by an individual blinded to patient outcomes using commercial enzyme linked immunosorbent assay (ELISA) kits (Quantikine, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Serum was stored at –80°C until analysis. Serum levels were recorded and Ang-2/Ang-1 ratio was calculated.

Statistical analysis:

Statistical analyses were performed using the software package SPSS/Win version 21 (SPSS Inc., Chicago, IL, USA). Qualitative data were presented as frequency and percentage. Chi square test was used to compare groups. Quantitative data were presented as median or

mean and standard deviation. For comparison between groups; *t*-test and Mann-Whitney test were used according the type of data. Correlation tests were performed using Spearman's rank test. A receiver operating characteristic (ROC) curve was used for determining cutoff values of Ang-2, Ang-1 and Ang-2/Ang-1 ratio as diagnostic parameters of septic shock development. Odds ratio was calculated for univariate risk estimation with 95% confidence interval. Kaplan-Meier test was used for overall survival analysis and the statistical significance of differences among curves was determined by log-rank test. A *p*-value equal to or less than 0.05 was considered statistically significant.

RESULTS

The present study was conducted on 20 males and 16 female patients with ages ranging from 18.0-72.0 years and a median of 38 years. Twenty-nine patients were diagnosed as AML and 7 patients had B-ALL. Seventeen out of the thirty-six patients developed septic shock.

Regarding inflammatory markers of sepsis, CRP was higher in patients who developed septic shock (mean level 89.35±43.92mg/dl) than patients with noncomplicated febrile neutropenia (mean level 53.32±22.37mg/dl) (p =0.006). Patients who developed septic shock had a lower MASCC score at onset of fever (ranging from 8 to 18 with a median of 16) compared to patients with non-complicated febrile neutropenia (ranging from 17 to 23 with a median of 21) (p-value=0.001).

Ang-2 serum level was higher in patients who developed septic shock compared to patients with non-complicated sepsis (p=0.034). Ang-1 levels were significantly lower in patients that developed septic shock compared to patients with noncomplicated FN (p=0.009). Therefore, the Ang2/Ang-1 ratio was higher in the septic shock-outcome group compared to patients with non-complicated FN (p<0.001; Table 1).

Estimation of the diagnostic accuracy of Receiver operating characteristics (ROC) curve showed that Ang-1 level <60ng/ml had 82.3% sensitivity and 68.4% specificity for the development of septic shock. Ang-2 level ≥600ng/ml had 84.21% specificity. Ang-2/ Ang-1 ratio >10 had 70.59% sensitivity and 89.47% specificity for the development of septic shock (Fig. 1).

The correlation between Ang-2, Ang-1 levels and different parameters was evaluated. Ang-2 level was positively correlated with CRP level (r=0.515, p<0.001) while Ang-1 was not correlated with CRP level. As regards MASCC score, Ang-2 showed negative correlation with MASCC score of the patients (r=–0.385,…p=0.020) while Ang-1 was positively correlated with MASCC score of the patients (r=0.399,…p= 0.016). Furthermore, higher Ang2/Ang-1 ratio was correlated with lower MASCC score (r=–0.509….p<0.001) and higher CRP levels (r=0.409….p=0.011). There were no significant correlations between Ang-2, Ang-1, or Ang-2/Ang-1 ratio on one side and either of complete blood count parameters, urea, creatinine, sodium, potassium, total bilirubin, direct bilirubin, albumin, ALT, AST, prothrombin time or activated partial thromboplastin time on the other side (Table 2).

The risk of development of septic shock was evaluated as regards the studied parameters. Significantly high risk was associated with the presence of comorbidity (p=0.046), hypokalemia (p=0.030), elevated CRP (p=0.013), high MASCC score (p=0.028), high Ang-2 (p= 0.046), and high Ang-2/Ang-1 ratio (p=0.007) (Table 3).

As regards mortality during the 28 days follow-up, better survival rates were observed in patients with Ang-1 level ≥60ng/ml (p= 0.035), and Ang-2 level <600ng/ml (p=0.003). However, Ang-2/Ang-1 ratio <10 was not significantly associated with better survival (p= 0.086) (Figs. 2-4).

Table (1): Angiopoitin-2, Angiopoitin-1, and Angiopoitin-2/angiopoitin-1 ratio in 36 acute leukemia patients with febrile neutropenia as regards development of septic shock.

Parameter	Septic shock		
	No (n=19)	Yes (n=17)	p**
Angiopoitin-2: pg/ml	554.79± 378.04*	970.59± 681.58	0.034
Angiopoitin-1: pg/ml	255.58± 304.94	55.65± 28.02	0.009
Ang-2/Ang-1	5.78± 5.05	17.55± 11.39	<0.001

* Mean ± SD.
** Statistically significant: p≤0.05.

Table (2): Correlation between Angiopoiotin levels, Angiopoiotin-2/Angiopoitin-1 ratio and clinical and laboratory parameters in 36 acute leukemia patients in relation to septic shock.

	Angiopoitin-2						Angiopoitin-1						Ang-2/Ang-1					
	All cases		No septic shock		Septic shock		All cases		No septic shock		Septic shock		All cases		No septic shock		Septic shock	
	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*
Hemoglobin level	–0.251	0.140	–0.029	0.906	–0.485	0.048	0.031	0.860	0.195	0.423	–0.213	0.412	–0.316	0.061	–0.149	0.543	–0.478	0.053
WBCs count	0.021	0.905	0.092	0.708	–0.119	0.648	–0.387	**0.020**	–0.280	0.245	–0.680	**0.003**	0.253	0.136	0.311	0.195	0.310	0.227
Neutrophils count	0.017	0.923	0.130	0.595	–0.062	0.813	–0.174	0.310	–0.134	0.584	–0.292	0.256	0.136	0.428	0.224	0.356	0.180	0.489
Platelets count	–0.029	0.865	0.204	0.403	0.121	0.643	0.118	0.494	0.047	0.850	0.052	0.844	–0.136	0.428	–0.011	0.963	0.031	0.907
Urea level	0.169	0.326	0.188	0.440	0.263	0.308	0.089	0.607	0.247	0.307	0.123	0.638	0.078	0.649	–0.144	0.555	0.138	0.598
Creatinine level	0.067	0.698	0.189	0.437	0.199	0.445	–0.062	0.720	–0.240	0.323	0.031	0.906	0.100	0.561	0.193	0.429	0.131	0.618
Na level	–0.145	0.399	–0.100	0.684	–0.072	0.783	0.15	0.383	–0.040	0.870	0.361	0.155	–0.143	0.405	–0.014	0.955	–0.333	0.192
K level	0.082	0.633	0.597	**0.007**	0.039	0.881	0.019	0.913	–0.400	0.090	0.009	0.972	0.051	0.768	0.520	**0.023**	0.117	0.655
CRP level	0.515	**0.001**	0.471	**0.042**	0.404	0.108	–0.091	0.597	0.081	0.742	0.167	0.522	0.418	**0.011**	0.072	0.769	0.451	0.069
MASCC score	–0.385	**0.020**	–0.130	0.596	–0.235	0.364	0.399	**0.016**	0.371	0.118	–0.206	0.428	0.053	0.761	0.283	0.240	–0.152	0.560

Significant values were typed bold.

Table (3): Univariate logistic regression analysis of clinical and laboratory markers of sepsis in 36 acute leukemia patients as regards the development of septic shock.

Parameter	Odds ratio	*p*	95% C.I.	
			Lower	Upper
Gender	1.222	0.765	0.327	4.565
Age	1.001	0.957	0.962	1.042
Type of leukemia	1.150	0.603	0.679	1.948
Comorbidity	9.819	0.046*	1.039	92.777
Hyponatremia	0.982	0.849	0.810	1.190
Hypokalemia	0.171	0.030*	0.035	0.841
Elevated CRP	1.039	0.013*	1.008	1.071
High ESR	1.007	0.652	0.976	1.040
Positive blood culture	4.000	0.053	0.981	16.311
High MASCC score	0.234	0.028*	0.064	0.852
High Angiopoitin-2	1.002	0.046*	1.000	1.0003
Low Angiopoitin-1	0.986	0.110	0.969	1.003
High Ang-2/Ang-1 Ratio	1.269	0.007*	1.069	1.507

C.I.: Confidence interval. * Significant.

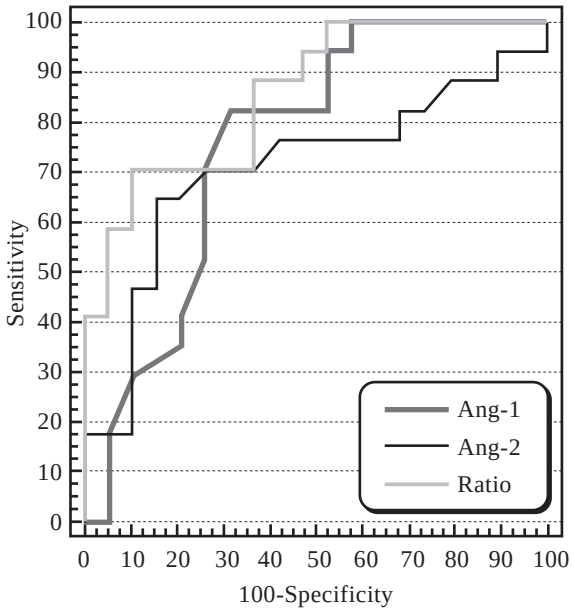

Fig. (1): Receiver operating characteristics curve of the development of septic shock in acute leukemia patients with febrile neutropenia as regards Ang-1 level <60pg/ml (specificity=68.4%), Ang-2 level >600pg/ml (specificity =84.2%), and Ang-2/Ang-1 ratio >10 (specificity=85.7%).

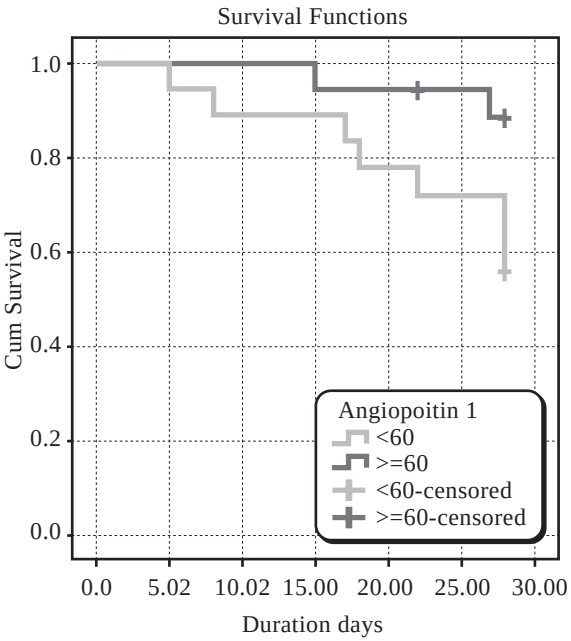

Fig. (2): Kaplan-Meier overall survival curve of acute leukemia patients with febrile neutropenia in septic shock according to Angiopoitin-1 level (p=0.035).

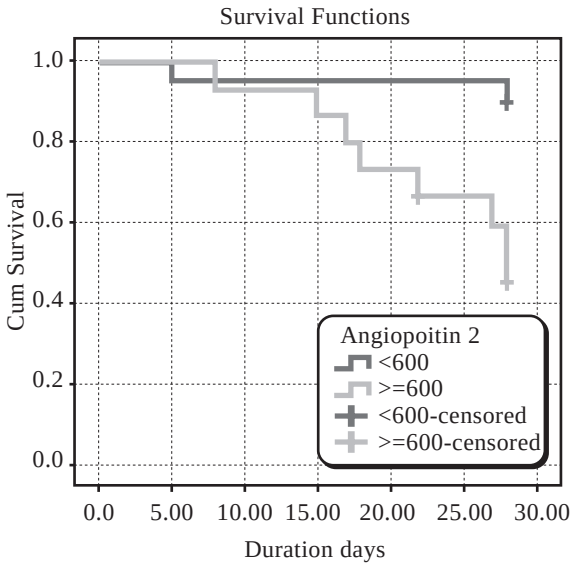

Fig. (3): Kaplan-Meier overall survival curve of acute leukemia patients with febrile neutropenia in septic shock according to Angiopoitin-2 level (p=0.003).

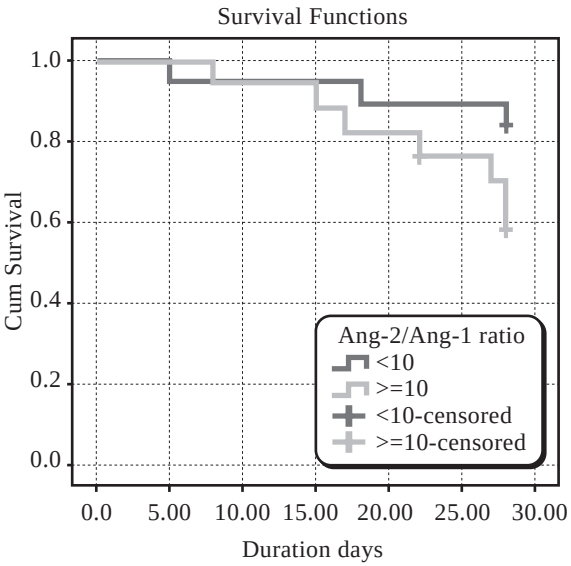

Fig. (4): Kaplan-Meier overall survival curve of acute leukemia patients with febrile neutropenia in septic shock according to Angiopoitin-2 / Angiopoitin-1 ratio (p=0.089).

DISCUSSION

Septic shock development is one of the worst complications of sepsis. Classically, it leads to multi-organ dysfunction syndrome and ends by death. Febrile neutropenic patients with hematologic malignancies present a high risk of septic shock **[14]**.

The most accepted risk stratification tool is the MASCC score. However, this score offers limited information for patients categorized as

high-risk (MASCC ≤21), the stage most acute leukemia patients with febrile neutropenia fit in. Accordingly, management of these patients remains a challenging situation for which the availability of informative biomarkers would provide better categorization and management **[15]**.

The aim of our study was to evaluate the role of Ang-1 and Ang-2 serum levels and Ang-2/Ang-1 ratio as endothelial barrier-stabilizing factors and as biomarkers of septic shock development in patients with acute leukemia suffering chemotherapy-induced febrile neutropenia.

Serum levels of Ang-2 were higher in patients who developed septic shock (p=0.009); besides higher Ang-2 was associated with higher CRP levels and lower MASCC score (p<0.001, p=0.020, respectively), while Ang-1 was lower in patients who suffered septic shock (p=0.034).

Ang-2/Ang-1 ratio was associated with septic shock development in our patients. Notably, it predicted septic shock development better than CRP, Ang-1, Ang-2, and MASCC scores.

In accordance with our results, Alves et al. **[16]** reported that acute leukemia patients who developed septic shock presented higher levels of Ang-2 and Ang2/Ang-1 ratio at the onset of fever compared to subjects with non–complicated sepsis (n=31). These levels correlated with sepsis severity scores.

In addition, Fiusa et al. **[17]** aimed to validate the use of these biomarkers in a large cohort of cancer patients having chemotherapy-associated FN. Ang-2 concentrations were increased in patients with septic shock compared to non-complicated FN, whereas an inverse finding was observed for Ang-1, resulting in a higher Ang-2/Ang-1 ratio in patients with septic shock. Moreover, multivariate analysis confirmed the value of Ang-2/Ang-1 ratio as an independent determinant of septic shock development and mortality at 28 days.

Our results showed that an Ang-2/Ang-1 ratio of 10 was the optimal cut-off value identified by the ROC procedure with a sensitivity of 70.59% and a specificity of 85.7% for the development of septic shock, while the study of Fiusa et al. **[17]** defined Ang-2/Ang-1 ratio of 5 as an optimal cut-off value which can be attributed to different sample size.

The role of angiopoietins as prognostic markers in sepsis has been evaluated by Ricciuto et al. **[18]** and Mankhambo et al. **[19]**. Ricciuto et al. **[18]** demonstrated that high plasma Ang-2 levels correlated strongly with morbidity during the course of ICU admission. They extrapolated that Ang-2 has a pathogenic role in sepsis and contributes to organ dysfunction, likely through endothelial activation and subsequent vascular leak, which leads to circulatory and renal compromise. In addition, they reported higher serially measured levels of Ang-2 in non-survivors relative to survivors (p=0.022). However, they did not report any association between admission levels of Ang-2 and 28 day mortality (p= 0.42).

Prior studies have reported an association between admission levels of Ang-2 and mortality in sepsis **[20,21]**. In agreement with this, high Ang-2 and low Ang-1 levels were found to be associated with higher mortality in the present study.

In conclusion, high Ang-2 level and Ang-2/Ang-1 ratio are predictive of septic shock development in acute leukemia patients with febrile neutropenia. High Ang-2 and low Ang-1 levels in febrile neutropenic patients are markers of higher mortality.

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